Physiological and behavioral effects of photosynthetically-active radiation on rosetip sea anemones *Condylactis gigantea:* Tolerance limits and growth

by

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Abstract

Irradiance is an abiotic factor that strongly affects the metabolism of endosymbiotic *Symbiodinium* within photosynthetic cnidarians such as rose tip sea anemones *Condylactis gigantea*. Fluctuations in the intensity of irradiance or photosynthetically active radiation (PAR) within and among environments can cause both behavioral and physiological changes to the holobiont, which consists of host sea anemones and their endosymbiotic *Symbiodinium*. Through laboratory experiments, we investigated: 1) anemone behavioral selection of PAR levels when placed in light tunnels (i.e., phototaxis), and 2) growth and physiological changes to the holobiont (both host anemone and *Symbiodinium*) when exposed to 3 PAR treatments (high, medium, and low) over 6 weeks. In terms of behavioral responses, we observed that anemones in light tunnels locomote to select locations exposed to a narrow range of PAR (~40-80 μmol photons m^{-2} s^{-1}, equivalent to irradiance at ~ 30 m depth on the open surface of some coral reefs). They exhibit this narrow range of irradiance selection when exposed to spatial variation in PAR levels over both broad (~10-300+ μmol photons m^{-2} s^{-1}) and fine scales (~20-140 μmol photons m^{-2} s^{-1}). Physiologically, anemones exhibit a more complex reaction to irradiance. Anemone growth did not differ among irradiance treatments, however anemones increased their tentacle crown surface area to wet mass ratio in response to low PAR. Alteration of this ratio may enhance light capture by endosymbiotic *Symbiodinium* in their tentacles when exposed to low PAR, allowing the algae to translocate more photosynthate to the host. We observed that the *Symbiodinium* rapidly acclimatize during the first two weeks of exposure to altered irradiance.

Specifically, significant changes in microalgal abundance and in chl α concentration per microalgal cell occurred in response to irradiance treatments. As large fleshy cnidarians, these sea anemones are able to respond both behaviorally and physiologically to alteration of PAR intensity, indicating an ability to acclimate to different irradiance environments, especially to low irradiance. These processes allow *C. gigantea* to occupy low-light microhabitats such as reef crevices, and also to occur over a wide depth range on Caribbean coral reefs. Based on these results, we recommend that conservation management of this species focus on protecting deep mesophotic reefs and highly rugose reefs where individuals may thrive in low-light environments. These types of habitats may serve as refuges for populations to reseed shallow reefs impacted by bleaching. We also recommend aquaculture conditions to enhance the growth and survival of this species in culture, thereby reducing the collection pressure on natural populations.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

TCSA	Tentacle Crown Surface Area
chl *a*	Chlorophyll *a*
PAR	Photosynthetically Active Radiation
MA	Microalgae Abundance
MI	Mitotic Index
SW	Salt water

Chapter I

Biological, ecological, and economical importance

of the rosetip sea anemone *Condylactis gigantea*

Importance of stress tolerance studies

Study of the physiological tolerance limits of organisms in response to environmental factors can contribute to predicting their distributional limits in ecosystems. The law of tolerance (Shelford 1913) states that organismal tolerance to both deficiency and excess determines the total range of resources or environmental factors (tolerance range) in which organisms can survive. The examination of tolerance ranges reveals the effects of variation in resource availability on organisms in their natural environments. Physiological response curves can reveal the optimal, suboptimal, supraoptimal, and lethal stress ranges of each type of resource affecting organisms. As environmental conditions deviate from an optimal range for a given factor such as temperature or light, organisms begin to express stress responses which increase as levels of the variable move further away from the optimum in either direction (i.e.: higher or lower than optimal levels). Quantifying stress tolerance contributes to understanding organismal distribution patterns in a given environment, by allowing visualization of the point at which a resource level becomes lethal (i.e.: either insufficient or excessive, leading to organismal death, Niinemets & Valladares 2008). These types of studies are becoming increasingly important for understanding organismal responses, as ecosystems worldwide are exposed to major anthropogenic changes in environmental variables such as temperature due to global climate change.

Metabolic physiology of sea anemones

Increasing anthropogenic stressors may play a key role in the ecophysiology and abundance of sea anemones. Among the abiotic factors affecting thermal stress tolerance thresholds, solar irradiance is perhaps the most significant (Lesser 2004). Solar irradiance consists of two portions, the visible spectrum (PAR, photosynthetically active radiation) that ranges from 400-700 nm in wavelength (Hoegh-Guldberg & Smith 1989, Brown et al. 2000), and ultraviolet radiation (UVR), that ranges from 290-400 nm and is not visible to the human eye. UVR may be divided further into UVA (320-400 nm) and UVB (290-329nm), with the relatively short wavelength UVB causing more damage to living cells than does UVA (Shick et al. 1996). Both UVR portions of the irradiance spectrum and PAR may negatively affect many photosynthetic anthozoans by inducing damage to PSII, photoinhibition, reduced photosynthetic efficiency, and bleaching at high levels (Goulet et al. 2005, Ferrier-Pagès et al. 2007, Frade et al. 2008, Hill & Scott 2011). Ultraviolet radiation is especially harmful; UVB causes structural damage to DNA by the formation of cyclobutane pyrimidine dimers (CPD), whose generation is largely UVB dose dependent (Lamare et al. 2007). This accumulation of CPDs can impair DNA polymerase function during replication and transcription, negatively influencing larval survival and reproductive success (Cubillos et al. 2015). These detrimental effects of UVB have been found in both invertebrates and vertebrates. For example, in the Antarctic sea urchin (*Sterechinus neumayeri*) CPD levels caused abnormal development, and in the coral *Agaricia agaricites* exposure to UVB caused an increase in coral larvae mortality (Gleason & Wellington 1995, Lamare et al. 2007). Early developmental stages in the Atlantic cod (*Gadus morhua)* show increased mortality and vitelum sac size reduction with exposure to UVB (Kouwenberg et al. 1999, Lesser et al. 2001, Browman et al. 2003).

Many types of sea anemones live in shallow marine habitats within the photic zone, and form symbioses with single-celled dinoflagellates in the genus *Symbiodinium* that live within their endodermal tissues. *Symbiodinium* also commonly associate with reef-building corals and other shallow marine invertebrates such as giant clams and nudibranchs. Sea anemones that live in shallow water and associate with *Symbiodinium* mitigate the negative effects of high irradiance in various ways, including through alteration of their microhabitat use. Dixon et al. (2014) found that sea anemones in very shallow environments occupied shaded reef holes and oriented their oral discs away from downwelling irradiance, thus exposing themselves to lower levels of irradiance and UVR than occurs in open exposed reef habitats. Another form of UV damage mitigation found in sea anemones is the production of mycosporine-like amino acids (MAA), which absorb both UVA and UVB at wavelengths between 310-315nm and 315-360nm respectively (Shick et al. 1996, Karentz 2001). These MAAs are photostable and have the ability to dissipate excess energy as heat, without the formation of reactive oxygen species (ROS) (Conde et al. 2000, 2004). Green fluorescent proteins also alter internal light absorbance when produced by some anthozoans (scleractininan corals), thus scattering light and protecting their *Symbiodinium* from excess irradiance (Salih et al. 2000). These mechanisms all may be important in protecting sea anemones and other cnidarians from harmful irradiance on coral reefs.

As ocean temperatures rise, sea anemones and other cnidarians have come under increasing threat of bleaching, in which they expel their *Symbiodinium* and ultimately may die if conditions persist (Hoegh-Guldberg 1999, Coles & Brown 2003). As the risk of cnidarian bleaching continues to increase due to temperature changes as a result of global climate change, as well as due to other biotic and abiotic stressors, some authors have suggested that the

mesophotic reef zone (30-150m depth below sea level) may act as a refuge for reef cnidarians (Slattery et al. 2011). Deep reef areas may be a feasible refuge from thermal stress, but as depth increases a loss in photosynthetically active radiation due to light attenuation also occurs. This can elicit stress in the symbiotic relationship between *Symbiodinium* and host cnidarians, because of a reduction in the metabolic input from photosynthesis.

The genus *Symbiodinium* contains nine clades (A-I), with multiple phylotypes found in each (Karim et al. 2015). A clade here is described as a grouping of phylogenetically distinct organisms, due to high DNA sequence variation among them (Santos et al. 2009). Regardless of which clade they belong to, *Symbiodinium* cells contribute significant amounts of fixed carbon in the form of photosynthate to their host organisms (McCloskey & Muscatine 1984). Some studies have estimated that the amount of photosynthate translocated to the host meets 90% or more of host metabolic needs (Muscatine et al. 1981, McCloskey & Muscatine 1984), but new research has shown that this may not be applicable to the carbon budget for all coral symbioses (Tremblay et al. 2013). Tremblay et al. found that to reach a rate of 90% translocation, corals had to be both under high irradiance, and have ample heterotrophic food available. Without this, the translocation rate of photosynthate dropped to between 71%-78% of host needs, depending on food availability and irradiance.

With a decrease in metabolic energy from *Symbiodinium* as light attenuates at depth, a shift in metabolic energy input towards heterotrophy occurs in many coral species (Palardy et al. 2005). Heterotrophic feeding in corals consists of a wide variety of particle sizes and nutritional sources, including dissolved organic material (DOM), particulate organic matter (POM), picoplankton, nanoplankton, and meso-macro-zooplankton (Houlbrèque & Ferrier-Pagès 2009). These nutrients acquired through heterotrophy increase both the amount of photosynthate

translocated from *Symbiodinium,* and the quality of photosynthate (Swanson & Hoegh-Guldberg 1998, Wang & Douglas 1998, Tremblay et al. 2013). Heterotrophy also allow corals to obtain nutrients not acquired through photosynthesis, mainly phosphorus and nitrogen but also carbon (Bachar et al. 2007). In sea anemones, feeding twice per week increases body mass and diameter by 50% and 25% respectively over ~2.5 months, compared to feeding only once per week or less which causes a loss of 30-50% mass and 15-30% diameter over the same time scale (Chomsky et al. 2004). Thus, sea anemones require heterotrophic food input, and flexibility in their metabolic input from heterotrophy versus autotrophy may allow them to become adapted to low-light conditions. Thus, a high rate of heterotrophic feeding combined with a low level of light input may be viable environmental conditions for the growth and reproduction (both sexual and asexual) of *Symbiodinium*-hosting anemones. However, if food acquisition is inhibited at depths where light exposure is low, this could cause a negative metabolic budget and subsequent organismal shrinkage and death.

Sea anemones acquire both carbon and nitrogenous organic products from byproducts of *Symbiodinium* photosynthesis and metabolism, and the resident *Symbiodinium* acquire the same elements from byproducts of the host anemone (Cleveland et al. 2010). This exchange of carbon and nitrogen from metabolic byproducts occurs also between anemonefish and their sea anemone hosts. Verde et al. (2015) showed that anemonefish gained these nutrients potentially by consuming the egesta, mucus, and/or tissues of anemones. They found a significantly higher amount of C^{13} and N^{15} isotopes in clownfish tissues when paired with anemones fed a diet of stable isotope labeled food. This same transfer of nutrients occurs from anemonefish to their host anemone and resident *Symbiodinium* as well (Cleveland et al. 2010).

Biology of Caribbean sea anemones, especially rosetip anemones Condylactis gigantea

Limited literature exists on the ecology and physiology of Caribbean sea anemones. One of the major coral reef anemones in the Caribbean Sea, corkscrew anemones *Bartholomea annulata*, can have highly dynamic populations (O'Reilly and Chadwick 2017). They utilize major broadcast spawning events twice each year to reproduce (Jennison 1981). Following recruitment, small individuals (< 25 cm^2 tentacle crown surface area; TCSA) grow rapidly; upon reaching medium size, the polyps (25.1-50 cm^2 TCSA) slow their growth, and large individuals (50.1-75cm^2 TCSA) remain static or grow at the slowest rate; populations have a high turnover rate with a lifespan of approximately one year (O'Reilly & Chadwick 2017).

Rosetip anemones (*Condylactis gigantea*) are also common Caribbean reef anemones, but even less is known about their recruitment, fecundity, and population dynamics. Sheridan et al. (2015) confirmed that *C. gigantea* is gonochoristic and has a 1:1 sex ratio in populations studied thus far, with only a few hermaphrodites present, and a single large spawning event occurs during May of each year. Individuals of *C. gigantea* are large, growing to > 30 cm diameter across the tentacle crown (Colin 1978), and inhabit the western Atlantic Ocean from the Gulf of Mexico to Florida and SE Brazil (Fautin 2013). With a range of 0-30m in depth below sea level, *C. gigantea* occurs in several types of nearshore zones and habitats including seagrass beds, lagoons, and coral reefs (Colin 1978, Briones-Fourzan et al. 2012). In parts of its range, *C. gigantea* is an economically-important organism as a major component of the aquarium trade. Due in part to over-collection, Florida populations of *C. gigantea* have declined since being identified as a species of greatest conservation need by the Florida Wildlife Conservation Commission (FWC) in 2005. This species was listed as "biologically vulnerable" by the FWC in 2012, and a ban on collection for 3 years was imposed (Sheridan et al. 2015). The ornamental

aquarium trade accounts for a large portion of anemone collection in Florida; between 1998-2012 roughly 2.1 million sea anemones were collected and sold bringing in approximately $1.6 million US dollars to the ornamental trade. Of the total number of sea anemones collected, 65% were *C. gigantea*, indicating that > 1.3 million individuals of this species were collected from Florida marine habitats over 15 years, or almost 100,000 per year (Sheridan et al. 2015).

Individuals of *C. gigantea* serve important ecological roles in Caribbean coral reef communities because they function as suspension feeders that contribute to benthic-pelagic coupling (Sheridan et al. 2015). They also act as hubs of a tripartite mutualistic network, in that they host at least 3 major types of eukaryotic organisms: single-celled endosymbiotic algae (*Symbiodinium* spp.) as well as exosymbionts composed of facultative fish and both facultative and obligate crustacean associates (Randall & Fautin 2002, Briones-Fourzan et al. 2012). This symbiotic system extends to 37 species of facultative reef fish associates (Arvedlund et al. 2006), 6 species of obligate crustaceans, and 10 facultative crustaceans (Briones-Fourzan et al. 2012).

The Pederson's shrimp (*Ancylomenes pedersoni*) is a Caribbean cleaner shrimp and an obligate symbiont of *C. gigantea*, as well as of its only other host, *Bartholomea annulata* (Criales 1984, Arvedlund et al. 2006, Briones-Fourzan et al. 2012). This cleaner shrimp is important ecologically, because it significantly reduces parasite loads on reef fish (Bunkley-Williams & Williams 1998, McCammon et al. 2010, Huebner & Chadwick 2012b). As *A. pedersoni* cannot survive without an anemone present, this symbiosis acts as a visual cue for reef fish when looking for a cleaning station (Huebner & Chadwick 2012a). As a visual cue *C. gigantea* is important in supporting indirect ecological effects across multiple tropic levels through the Pederson's shrimp (Sheridan et al. 2015). This participation in a potentially large

mutualistic network involving many species of client fishes is yet another reason why *C. gigantea* is an ecologically important species throughout its natural range.

Condylactis gigantea has been found to host several clades of *Symbiodinium* including A, B, and C (LaJeunesse 2002, Savage et al. 2002, Venn et al. 2008). In Bermuda, some individuals host mixed infections of clades A and B, with possible seasonal temporal variation of the dominant clade (Venn et al. 2008). This mix of infections has been studied only in Bermuda, but could be a major factor in the adaptation of *C. gigantea* to reef habitats in other parts of the Western Atlantic including the Caribbean Sea.

Little is known about how variation in irradiance affects the growth and physiology of Caribbean sea anemones, including *C. gigantea*. Due to their ecological importance and wide distribution on Caribbean reefs, it is important to better understand how this variation affects the physiology and behavior of *C. gigantea*.

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Chapter II

Effects of irradiance on the physiology and behavior of rosetip sea anemones *Condylactis gigantea* and their microalgal symbionts

Introduction

The law of tolerance states that organismal resilience to both deficiency and excess determines the total range of resources or environmental factors (tolerance range) in which organisms can survive (Shelford 1931). As environmental conditions deviate from an optimal range for a given factor such as temperature or light, organisms begin to express stress responses which increase as levels of the variable move further away from the optimum in either direction (i.e.: higher or lower than optimal levels). Stress tolerance analysis contributes to understanding organismal distribution patterns in a given environment by allowing visualization of the point at which a resource level becomes lethal (i.e.: either insufficient or excessive, leading to organismal death, Niinemets & Valladares 2008). These types of studies are becoming increasingly important for understanding the responses of marine organisms as coastal ecosystems worldwide are exposed to major anthropogenic changes in environmental variables predominately resulting from global climate change.

Anthropogenic stressors are playing an increasingly significant role in the ecophysiology and abundance of sea anemones. As ocean temperatures rise, sea anemones and other anthozoans

such as stony corals have come under increased threat of bleaching. Among the abiotic factors that affect their thermal stress tolerance thresholds, solar irradiance is perhaps the most significant (Lesser 2004). High levels of both portions of the solar irradiance spectrum (photosynthetically active radiation [PAR] and ultraviolet radiation [UVR]) may negatively impact photosynthetic anthozoans and compound their susceptibility to bleaching through photoinhibition, damage to Photosystem II (PSII), and reduced photosynthetic efficiency (Hoegh-Guldberg & Smith 1989, Brown et al. 2000, Goulet et al. 2005, Ferrier-Pagès et al. 2007, Frade et al. 2008, Hill & Scott 2011). Tropical anthozoans can mediate the effects of irradiance stress by sheltering in low-light refuges, or by positioning themselves relatively deep in the water column in the mesophotic reef zone (30-150m depth; Slattery et al. 2011). However, decreases in PAR due to light attenuation at depth also reduce autotrophic metabolic input to symbiotic reef anthozoans from their microalgae *(Symbiodinium)*. As such, the optimal PAR level for many symbiotic anthozoans may occur at some mid-range of depths below sea level (Dixon et al. 2013). Understanding the PAR tolerance ranges like those for coral reef sea anemones, is needed because the threat of with mass bleaching events in shallow tropical waters (Hill & Scott 2011). The ability of shallow tropical anemones to survive in environments with low PAR such as on deep reef slopes and mesophotic reefs may determine their ability to survive as oceans change.

Rosetip sea anemones (*Condylactis gigantea*) are important both ecologically and economically in Caribbean marine ecosystems, but almost nothing is known about their population dynamics or environmental tolerances. In parts of their geographical range, individuals of *C. gigantea* are a major component of the ornamental aquarium trade. In Florida alone, > 1 million individuals were collected from Florida marine habitats over ~15 years (1998-2012; almost 100,000 per year; Sheridan et al. 2015). The Florida Wildlife Conservation

Commission (FWC) identified them as a species of greatest conservation need in 2005, but they continued to decline and were listed as "biologically vulnerable" in 2012; a collection ban then was imposed for 3 years (Sheridan et al. 2015) and was subsequently extended (http://myfwc.com/research/saltwater/codes/prohibited-species/giant-caribbean-anemone/), probably due to lack of population recovery. Individuals have been collected extensively for the ornamental trade also in Puerto Rico (LeGore et al. 2005) and Haiti (pers. comm., Blue Zoo Aquatics).

Individuals of *C. gigantea* serve multiple ecological roles in Caribbean coral reef communities, acting as hubs of a multi-level mutualistic network as do other large reef anemones (Roopin & Chadwick 2009, Cantrell et al. 2015). They host several types of eukaryotic organisms: endosymbionts comprised of A, B, and C clades of microalgae (*Symbiodinium* spp., Perez et al. 2001, Savage et al. 2002, Karako-Lampert et al. 2005). Polyps of *C. gigantea* also host exosymbionts composed of 37 facultative fish species (Arvedlund et al. 2006), 10 facultative crustaceans, and 6 obligate crustaceans (Randall & Fautin 2002, Briones-Fourzan et al. 2012). A clade here is described as a grouping of phylogenetically distinct organisms, due to the high DNA sequence variation. (Santos et al. 2009). Pederson's shrimp *Ancylomenes pedersoni* are obligate associates of *C. gigantea* (Criales 1984, Arvedlund et al. 2006, Briones-Fourzan et al. 2012) and function as the major crustacean cleaner of fish parasites in the Caribbean Sea, causing significant reductions of parasite loads on a wide variety of reef fishes (Bunkley-Williams & Williams 1998, McCammon et al. 2010, Huebner & Chadwick 2012b, Titus et al. 2017). The sea anemone hosts of *A. pedersoni* are visually conspicuous and thus act as visual cues for reef fishes to locate cleaning stations (Huebner & Chadwick 2012a). In their role as major hosts of cleaner shrimp, *C. gigantea* and other reef anemones likely cause indirect

ecological effects across multiple tropic levels on coral reefs (Cantrell et al. 2015, Sheridan et al. 2015).

As threats to coral reefs and other coastal ecosystems increase due to anthropogenic stressors including climate change and overfishing, the likely continuing declines in populations of these *C. gigantea* and other sea anemones may have far-reaching negative effects on Caribbean marine ecosystems. Yet, almost nothing is known about their environmental tolerances, except that individuals occur over a depth range of 0-30m in several types of nearshore habitats including seagrass beds, lagoons, and coral reefs (Colin 1978, Briones-Fourzan et al. 2012). Information about the physiological tolerances of *C. gigantea* for major factors that control their distributional patterns, including irradiance, is needed to provide a scientific basis for the conservation management of this species. Here we determine the optimal levels photosynthetically active irradiance (PAR) selected by *C. gigantea*, as well as their behavioral and physiological responses to PAR. We report results from two types of laboratory trials: (1) behavioral experiments to elucidate the ability of individuals to locomote in relation to irradiance (phototaxis) and thereby to select optimal exposure levels, and (2) physiological experiments to assess how irradiance influences the growth of *C. gigantea* as well as characteristics of their resident *Symbiodinium*.

Methods

Organismal collection and culture

The present study was conducted at Auburn University over 1.5 years (August 2016-February 2018). Individuals of *C. gigantea* were acquired through a commercial vendor (Blue

Zoo Aquatics, Hawthorne, California), who received them from collection sites in Haiti. Anemones were acquired in 5 separate orders: 10 individuals arrived in February 2017, 14 in March 2017, 12 in May 2017, 30 in September 2017, and 22 in October 2017. Mortality of 2 anemones during shipping resulted in 86 anemones arriving alive to the laboratory, with 12-30 individuals present in the laboratory at any one time. Anemone mortality occurred in the laboratory due overheating from malfunction of a building air conditioning unit (N = 24), and from unknown causes (N = 3). An additional 11 anemones were cultured as extra animals to use in experiments in case of further mortality, but were not used in the present study (total N = 86 anemones: 48 used in experiments, 11 cultured as extras, and 27 that suffered mortality before use in experiments).

After arrival to the laboratory, all 86 anemones were cultured in closed-system tanks (4 anemones per tank x 6 tanks = 24 anemones at any one time, with an additional tank for culture of 2-12 extra anemones, see above). Three closed system tank setups were used, each consisting of 2 70-L culture tanks (each 77 cm length x 32 cm width x 33 cm height) connected to one 70-170L sump tank (2 tanks per system x 3 systems = 6 tanks). An additional 70L tank was used for the culture of extra anemones not used in the experiments; this tank had a simple hang-on back canister filter. The paired tank setup was employed to create a large volume of water for each culture tank system, which enhanced water quality stability. Tank salinity (~34 ppt) and temperature (~25°C) were monitored daily and adjusted as needed to mimic natural conditions, similar to methods used for previous long-term culture of tropical sea anemones in the same laboratory (Roopin & Chadwick 2009, Cantrell et al. 2015). Each culture tank was illuminated on a 12:12hr light:dark cycle, using two Light Emitting Diode (LED) fixtures (Galaxyhydro 165W) hung over the tank and spaced evenly to distribute light throughout the tank. Each culture

tank also contained a 1-2 cm layer of coarse gravel, and each sump tank had a water pump, protein skimmer, and bioballs for filtration.

During routine culture, the lights were adjusted so that anemones were exposed to ~100-150 μmol photons m^{-2} $s^{-1}$of photosynthetically active radiation (PAR), similar to PAR levels at ~ 15-25 m depth on Caribbean coral reefs (Lesser 2000), achieved by adjusting the dimmer on the LED lights to 40%. PAR was measured at the upper surface of each anemone using a QSL-2001 Scalar PAR Sensor (Biospherical Instruments, San Diego, CA, USA). To facilitate individual anemone identification, each anemone was cultured in a separate tank section, created by positioning dividers made of rigid plastic grating (32 cm width x 35 cm height, extending above the tank top; 1.5 x 1.5 cm grating holes, Plaskolite) at ~20 cm distances along the length of the tank (4 sections per tank). To prevent anemone movement through the dividers, fine plastic mesh (4 x 4 mm holes, Yarnology #5 mesh) was attached to each divider using plastic zipties, to cover 12 cm height above the gravel at the tank bottom. A large rectangular (25 x 8 cm) hole was cut into each divider slightly below the water surface to facilitate water flow throughout the tank. Two spigots, one at each end of the culture tank, supplied alternating water flow from the sump. Each anemone was fed individually 1x per week with a $1cm^3$ piece of raw shrimp. This feeding rate was used so that individuals received heterotrophic nutrients, but were not fully satiated with food, as occurs for sea anemones during 2x per week feeding or more frequently (Chomsky et al. 2004). This limited feeding rate caused the anemones to rely more on autotrophy by their microalgae, and allowed us to more clearly detect their responses to changes in autotrophy, through irradiance effects on microalgal photosynthesis (after Roopin & Chadwick 2009)

During culture, anemone body size was measured every 2-3 weeks as tentacle crown surface area (TCSA, after Cantrell et al. 2015, O'Reilly & Chadwick 2017). All animals were cultured for at least 1 week up to several months prior to use in experiments.

Phototaxis behavior

Experiments were performed to determine if anemones exhibited phototaxisalteration of their positions in relation to a gradient of irradiance, by either locomoting along the substrate or floating through the water column). In the initial phototaxis experiment during May 2017, an irradiance gradient was created to form a light tunnel in each of the 6 culture tanks (see above; after Yamashiro & Nishira 1995). Aquarium lights all were set to 100% output, and then modified using light filters to form the same type of light tunnel in all 6 tanks (Fig. 1a). A combination of window screen, plastic mesh (Yarnology #5 mesh), and black cloth was used to create the light filters, which then were laid over the top of each tank. Window screen and black cloth were attached to 12" bamboo wooden skewers to create a basic light filter, then plastic mesh or window screen was layered onto the basic filters to vary the irradiance level across the tank. The light filters (sections of screen and cloth each 35 x 17cm, which were the upper dimensions of each of the 4 tank sections, see above) were placed on top of a 1.5 cm strip of plastic grating that was attached to the inside rim of each tank. This was done to position the light filters ~ 4.5 cm above the water surface and 16-18 cm below the light fixture. The rigid plastic grating tank dividers then were removed from each culture tank, and black cloth was layered over one of the light filters (17 x 35 cm area) so that ¼ of the tank length at one end received low irradiance (~ 9 to 100 μmol photons m^{-2} s^{-1} PAR). Fewer layers were placed over the 2 light filters on top of the mid-tank sections, so that the middle half of the tank (~35 x 35 cm

area) received a gradient of medium-low to medium-high irradiance (~ 100-250 μmol photons m^{-2} s^{-1}). No light filter was placed over the far tank end so that ¼ of the tank (17 x 35 cm area) received high irradiance (250-400 μmol photons m^{-2} s^{-1}). Irradiance levels were measured at a height of ~ 4 - 6 cm above the tank bottom, near the upper surfaces of the anemones inside the tanks. Due to the scattering of irradiance in water, these 4 overhead filter conditions created a fairly continuous gradient of irradiance from one end of the tank to the other (ie: a light tunnel, ranging ~ 9-400 μmol h m^{-2} s^{-1} PAR along the 75 cm length of each tank; Fig. 1a). A plastic measuring tape was attached along the outside of each tank to mark anemone positions along the light tunnel. The direction of the tunnel gradient (dark to light) was assigned randomly to each tank, beginning at the right or left end. Creation of the light tunnels required only a few hours per tank, and did not appear to adversely affect the resident anemones which remained inside the tank.

Anemones then were assigned randomly among the 6 tanks, to create new combinations of 4 anemones per tank x 6 tanks (N = 24 anemones total). Each randomized anemone was removed from its original culture tank and placed, unattached, in the center of the water column in its newly-assigned experimental tank. Anemones in each experimental tank were identified individually during the phototaxis experiment based on variation in their body sizes and in column and tentacle coloration, including the presence or absence of rose tips on their tentacles.

During the first day of the experiment, the 24 anemones were allowed to settle and attach to the substratum in each tank. At ~4 hours after the anemones were deposited into their experimental tanks, their distance from the dark end of the tank as well as, PAR levels recorded. Then beginning on Day 1 (the following day), additional information was collected for each anemone during each day for 1 week: (1) distance from dark end of the tank (measured by taking

photographs through the front of the tank); (2) level of tentacle expansion, quantified as percent expansion at five levels: 100% (completely expanded), 75% (mostly expanded), 50% (half expanded), 25% (mostly contracted), 0% (completely contracted; after Levy et al. 2006); (3) oral disk orientation, measured as the angle of orientation of the oral disk in relation to the major direction of irradiance reaching the anemone: ranging from 0° (oral disk perpendicular to irradiance; ie: irradiance mostly reaching the upper surface of the anemone on the oral disk and tentacles) to 90° (parallel to the irradiance; irradiance mostly reaching one side of the anemone along the column); and (4) irradiance level (PAR) at 1-2 cm above the center of the tentacle crown.

At the end of 1 week, light filters were removed and the tank lights were reset to ~100-150 μmol photons m^{-2} s^{-1}, with the vertical tank dividers being re-inserted to separate each anemone into a different tank section, thus return them to standard culture conditions.

Based on the results of this initial experiment and to determine finer-scale anemone responses over both a shorter initial duration and a narrower range of irradiance levels, a second phototaxis experiment was conducted 9 months later in February 2018. All anemones for this experiment were taken from the previous 6-wk irradiance experiment (N=15). These anemones were cultured at 100-150 μmol photons m^{-2} s^{-1} for 10 weeks, to allow all anemones to recover from the initial light trials, and to have the same baseline of physical characteristics. The second experiment was conducted using the same methods as the first one, except that it examined anemone responses: (1) more frequently during the first 12 hours (at 15 min, then 1, 3, 5, 7, 9, and 11 hours in the first day) then daily for 1 week, and (2) over a narrow range of irradiance levels within each light tunnel (20 μmol photons m^{-2} s^{-1} PAR at one end of the tank to only 140 μmol photons m^{-2} s^{-1} PAR at the other end).

Effects of irradiance on anemone body size and Symbiodinium characteristics

To determine how anemone body size and *Symbiodinium* characteristics varied with irradiance level over several weeks, 6-wk laboratory experiments were conducted in which each anemone was exposed to 1 of 3 irradiance levels (= PAR at 3 depth ranges below sea level on coral reefs; Lesser 2000, Dixon et al. 2014): (1) Low (~50µmol photons m^{-2} s^{-1}; ≥ 30m depth), (2) Medium (~150µmol photons m^{-2} s^{-1}; ~15-25m depth), and (3) High (300+µmol photons m^{-2} s^{-1}; ~0-10m depth). Due to technical limitations of the light fixtures, very high PAR levels > 600 µmol photons m^{-2} s^{-1} were not attainable, even though they occur at or near the sea surface on some coral reefs (Lesser 2000, Dixon et al. 2014).

To attain the PAR level for each treatment, all light fixtures were set to 100% output (Galaxyhydro 165W), and light filters using shade cloth squares were fashioned for the medium and low treatments, similar to those created for the phototaxis experiments above. Each irradiance treatment was assigned randomly to one of the 4 sections in each of 6 tanks (1 anemone per section x 4 sections per tank x 6 tanks = 24 anemones total; Fig 1b). The first 6-wk experiment used the same anemones from the first 1-wk experiment. Because there were only 3 irradiance treatments, there were 8 anemones per treatment x 3 treatments = 24 anemones total, with at least one replicate of each treatment in each tank. Individual anemones were assigned randomly to each treatment, similar to the process for the phototaxis experiments. The plastic tank dividers were retained to prevent the anemones from moving among treatments within each tank, and were cleaned each 2-3 weeks with a rigid brush to prevent algal buildup. For each tank a 25% water change was performed after 4 weeks to maintain water quality.

The first 6-wk experiment began in June 2017. The following variables were measured at 0, 2, 4, and 6 weeks after treatments began: anemone tentacle crown length and width (for

calculation of TCSA; measured using a plastic ruler to the nearest millimeter), microalgal cell abundance (MA), chlorophyll *a* concentration per microalgal cell (chl *a*), and mitotic index (MI; (Cantrell et al. 2015, O'Reilly & Chadwick 2017). To quantify relationships among body size parameters, whole animal wet mass (WM) also was measured before the start of the experiment (~5 days beforehand, to allow individuals to recover and expand fully before the experiment began). Each anemone was removed from its home culture tank, gently massaged to induce contraction and expulsion of water from the gastrovascular cavity, lightly dabbed with a paper towel to remove excess water, and placed in a weigh boat on an electronic scale. Each animal was out of its tank for < 2 mins and appeared to recover fully from this process, as indicated by complete expansion and attachment to the tank substrate within ~ 3 hours (Chomsky et al. 2004, Cantrell et al. 2015). Wet mass was also measured at the end of each 6-wk experiment.

To obtain *Symbiodinium* measurements, one tentacle was selected haphazardly from the inner tentacle crown of each anemone, and 1-2 cm of tentacle tip was removed using scissors. Wet mass was taken of each tentacle tip before it was homogenized in 1ml of SW. This solution was then placed in a 2ml microcentrifuge tube (VWR) and centrifuged at 5g for 5min at ~ 24°C. Supernatant was removed, and the algal pellet resuspended in 1ml of SW and vortexed. This process was repeated, and 0.5ml of the final solution of suspended cells was placed in a separate 2ml microcentrifuge tube for chl *a* analysis. Samples were diluted and microalgal abundances and mitotic index were counted using a Hausser Scientific hemocytometer under 400X magnification of a phase contrast microscope. Five subsamples of microalgal counts were taken from each sample. Microcentrifuge tubes set aside for chl *a* analysis were centrifuged, and the supernatant removed. The algal pellet was then suspended in 1ml of 90% acetone and left overnight at 4°C for chlorophyll extraction (after Roopin & Chadwick 2009, Cantrell et al.

2015). The following day each suspension was centrifuged at 5g for 5 min and the supernatant placed into a cuvette for spectrophotometric analysis using a Genyses 5 spectrophotmeter. Chlorophyll *a* content was acquired using equations from Jeffrey & Humphrey (1975).

The 6-wk experiment was repeated starting in November 2017, using the same methods applied to 24 anemones that were different from those used in the first 6-wk experiment, so as to increase the experimental sample size.

Statistical analyses

In the experiment on anemone growth and physiological changes, differences in effects of irradiance level among all treatments were assessed using a mixed effect general linear model. The model included fixed effects for time, treatment, and the time x treatment interaction, as well as a random effect for individual anemone. Statistical analyses were run utilizing the lme function from the nlme package (Pinheiro et al. 2018) in R (R Core Team 2016). All results are reported as means ± 1 confidence interval unless described otherwise.

Results

Phototaxis behavior

During the first phototaxis experiment, most individuals (87.5%) of *C. gigantea* (N = 24) settled to the tank bottoms and attached to the rubble substratum within the first 4 hours after introduction to the light tunnel tanks. Three individuals remained suspended in the water column

throughout the experiment and did not attach to the substratum; they were subsequently excluded from analysis.

Anemones initially attached to a wide range of locations in the tanks, and were exposed to irradiance levels that ranged from very low (7 µmol quanta m^{-2} s^{-1} PAR) to high (~300-430 µmol quanta m^{-2} s^{-1}; Fig. 1a). The anemones varied over time in their exposure to irradiance, as they moved around the tanks over 1 wk (Fig. 2). Most individuals (61.9-71.4% depending on the day) selected tank locations that received relatively low irradiance (<100 µmol quanta m^{-2} s^{-1} PAR), while only a few individuals (14.29-19.05% depending on the day) selected locations exposed to high irradiance (>150 µmol quanta m^{-2} s^{-1}). Peak anemone abundance (28.57-61.9%) consistently occurred at 50-100 µmol quanta m^{-2} s^{-1} on all days during the week of exposure (Fig. 2).

During the second phototaxis experiment, individuals were exposed to a narrower range of irradiance (14-140 µmol quanta m^{-2} s^{-1}) and observed more frequently (at least hourly during the first day). All individuals (N = 15) settled to the tank bottoms and attached to the substratum within 15 minutes after introduction to the light tunnel tanks. Most (60%) initially selected locations exposed to low irradiance (20-60 µmol quanta m^{-2} s^{-1}) and remained there throughout the first day (46.6-73.3% of individuals, depending on hour during the day; Fig. 3). On subsequent days, their exposure to irradiance varied somewhat as they moved around the tanks, but the majority (61.54 %) remained at < 60 µmol quanta m^{-2} s^{-1} at the end of the week of exposure (Fig. 4). Peak anemone abundance consistently remained within a narrow band of low irradiance (~40-60 µmol quanta m^{-2} s^{-1}), with few individuals occurring at relatively high irradiance (>100 µmol quanta m^{-2} s^{-1}).

Effects of irradiance on host body size and microalgal characteristics

In the experiment on effects of irradiance on anemone growth and microalgal characteristics, the 2 main measures of anemone body size (TCSA and WM) varied widely among individuals within each treatment (Figs. 5 and 8). Prior to the treatments being applied (Week 0), TCSA did not differ significantly among the 3 treatments (all $p > 0.15$, Table 1). During the first week of exposure to treatments, there was a significant interaction effect between time and treatment, such that the difference in TCSA between low and high irradiance decreased by 40.71 (± 40.67; 95% CL) cm^2 from Week 0 to Week 2 ($p = 0.048$ interaction term; Table 2, Fig. 5A). By Week 2, anemone body size under low irradiance was 36.69 (± 38.58; 95% CI) cm^2 larger than that under high irradiance, but this difference was not statistically significant ($p = 0.07$). Differences in TCSA among the treatments did not change significantly in the medium and low treatments between Week 0 and Week 6 ($p = 0.16$ and 0.10, respectively, Fig. 5A).

Wet mass, similar to TCSA, also did not differ significantly among the treatments at Week 0 (all $p > 0.48$). However in contrast to TCSA, sea anemone body size as measured in wet mass decreased significantly between the start and end of the experiment, at all 3 levels of irradiance (all $p < 0.0011$). The change in anemone body mass in all treatments combined ranged from -40.9g to 6.4g (i.e., major shrinkage to slight growth), which was equivalent to a range of -79.2 to 41.3% change in body mass over 6 weeks. Similar to TCSA, anemone body size as measured in wet mass varied widely among individuals, but did not vary significantly among treatments after 6 weeks (Fig. 7).

As expected from the pattern of relatively constant TCSA but decreasing WM over time, the ratio of TCSA:WM increased substantially under low irradiance, by 42.15% over 6 weeks. In contrast, this ratio decreased slightly in both the medium and high irradiance treatments (by 12.0% and 19.7%, respectively). By the end of the experiment at Week 6, anemones exhibited a significantly higher ratio of TCSA:WM under low irradiance than under medium irradiance ($p = 0.04$, Table 1, Fig. 5B).

Microalgal abundance also did not differ significantly among treatments during Week 0 (all $p > 0.19$, Table 1), but varied widely (10-fold) among individual anemones within each treatment throughout the experiment (overall range = $4.9x10^7$ - $4.7x10^8$ cells per gram wet mass). By Week 2, we observed a significant difference between the high and low treatments, in which the abundance of *Symbiodinium* under low irradiance was $6.92x10^7$ ($\pm$ $6.23x10^7$; 95% CI) cells $gram^{-1}$ wet mass higher than under high irradiance ($p = 0.03$). Then during subsequent sample periods, microalgal abundances did not differ significantly among the treatments (Table 1, Fig. 6).

The mitotic index (MI; cell division rate) of the *Symbiodinium* cells likewise did not differ significantly among treatments during Week 0 (all $p > 0.55$, Table 1). Similar to microalgal abundance, mitotic index varied widely among individual anemones throughout the experiment (0 – 15.83% of microalgal cells were observed to undergo cell division at any one time), and did not differ significantly among irradiance treatments (Table 1, Fig. 7). MI decreased from ~7-8 % of cells dividing prior to the application of treatments, to only ~3-4.5% of cells dividing after 6 weeks in all treatments (all $p < 0.009$), but these decreases were not significantly different among treatments (*i.e.*, time x treatment interaction; all $p > 0.40$)

Before the start of the experiment at Week 0, anemones assigned to the low irradiance treatment contained 0.20 (± 0.15; 95% CI) pgchl *a* per cell less than in those assigned to high irradiance ($p = 0.009$, Table 1). The difference between low and high irradiance treatments significantly decreased by 0.33 (± 0.19; 95% CI) pgchl *a* per microalgal cell during the first week of treatments ($p = 0.0005$, interaction term, Fig. 6B, Table 2), due to increasing chl *a* concentration under low irradiance and decreasing concentration at high irradiance. The effect of treatment was significant at Week 4, in that the chl *a* concentration was 0.19 (± 0.15; 95% CI) pgchl *a* higher per microalgal cell in anemones exposed to low vs. high irradiance ($p = 0.01$, Table 1). The same temporal trend for time 2 occurred again in time 6, with no significant effect of treatment (Fig. 6B, Table 1).

Prior to the application of treatments at Week 0, sea anemones in the medium irradiance treatment contained 0.19 (95% CL; ± 0.15) pgchl *a* per algal cell less than at high irradiance ($p = 0.012$, Table 1, Fig. 6B). The difference between anemones in the medium and high irradiance treatments decreased by 0.28 (± 0.18; 95% CI) pgchl *a* per algal cell between Weeks 0 and 2 ($p = 0.003$, interaction term, Table 2), due to an increase in chl *a* under medium irradiance and a decrease under high irradiance. However, this significant interaction did not persist between Weeks 2-4 and 4-6 ($p = 0.22$ and 0.52, respectively, Fig. 6B, Table 2), because chl *a* levels under medium irradiance decreased to similar levels as under high irradiance during Weeks 4 and 6 ($p = 0.76$ and 0.62, respectively, Fig, 5B, Table 2). Chl *a* concentrations differed between the anemones exposed to low vs. medium irradiance during Week 4, when the anemones under low irradiance contained 0.22 (± 0.15; 95% CI) pgchl *a* per microalgal cell higher than for the anemones exposed to medium irradiance ($p = 0.006$, Table 1). The combined changes in microalgal characteristics indicated that the anemones increased both the abundance of

Symbiodinium in their tentacles and the concentrations of chl *a* per microalgal cell, when exposed to low but not high irradiance, especially during the first few weeks of exposure (Fig. 6).

Discussion

Our results demonstrate that both behavioral and physiological changes occur in *Condylactis gigantea* in response to variation in irradiance intensity. Past studies on effects of irradiance in photosynthetic cnidarians centered around how changes in this abiotic factor impacted the photosynthetic efficiency, output, and thermal tolerance of the host organisms and their symbiotic dinoflagellates (Muscatine et al. 1981, Shick et al. 1996, Brown et al. 2000, Lesser 2004, Tremblay et al. 2013). This is the first study to report how varying irradiance intensity affects the anthozoan holobiont on both behavioral and physiological levels.

Behavioral and physiological changes in relation to irradiance

Previous studies on sea anemone phototaxis found that individuals harboring microalgal symbionts showed both positive and negative phototaxis relating to the intensity of irradiance (Zahl & McLauglin 1959, Pearse 1974a). Our findings support these studies, and expand on them by showing a specific range of irradiance which *C. gigantea* anemones apparently select, at ~20-120 μmol photons m^{-2} s^{-1} (Figs. 1 and 3). The fine scale phototaxis experiment revealed further specificity, in that these anemones select a low light environment exposed to a narrow range of ~40-60 μmol photons m^{-2} s^{-1}, equivalent to irradiance levels at ~30m depth below sea level on some coral reefs (Lesser 2000). However, irradiance can vary dramatically within a

given depth below sea level, depending on water clarity in different types of coral reef habitats. While individuals of *C. gigantea* occur on the deep reef slope at 30 m depth, they are more commonly found in shallower habitats ranging 1-20 m depth (Table 3). The reason for this apparent discord between our findings and the recorded depth distribution of *C. gigantea* may be due to multiple factors. One factor is the cyclical nature of irradiance intensity throughout the day. Starting with sunrise, PAR levels increase until they reach a maximum at midday, then decrease until sunset. This daily cycle delivers high intensity irradiance to sea anemones over only a brief period, surrounded by exposure to moderate and low irradiance over a much longer period each day (Lesser 2000). Because of varying proportions of irradiance intensity throughout the day, even in shallow waters sea anemones are exposed to relatively low irradiance during most of each day. In conjunction with this variation in irradiance intensity, the physiological acclimation responses of *C. gigantea*, such as reduced microalgal abundance, chl a levels, and tentacle crown surface area, may enable individuals to live in shallow environments. Furthermore, the soft bodied nature of these cnidarians allows for tissue expansion and contraction as a mechanism to alter their body exposure to irradiance, and is most probably linked to their relationship with resident *Symbiodinium* (Pearse 1974b). Behavioral habitat selection can alter the irradiance environment of sea anemones; in the Red Sea, shallow individuals of bulb-tentacle sea anemones *Entacmaea quadricolor* inhabit shaded reef holes thereby reducing their exposure to irradiance (Dixon et al. 2014). Similarly, individuals of *C. gigantea* sometimes attach their bases in crevices or holes on coral reefs (N.E. Chadwick, pers. comm.). All of these processes combined may help to explain how rosetip anemones protect themselves from high light in the field, and explain differences between the results of our laboratory experiments and observations of their depth distributions on coral reefs.

Few studies have quantified variation in the abundance of *C. gigantea* with depth below sea level. Scattered reports in the literature and from unpublished field observations reveal that individuals of this species mostly have been observed at shallow (~1-10m) depths on coral reefs, but that individuals also have been seen up to 37 m deep (Table 3). The highest recorded abundances of *C. gigantea* are reported from patch reefs at only 1-4 m depth in Akumal, Mexico, where up to 3 individuals occurper 10 m^{-2} (Colombara et al. 2017; Table 3). In contrast, their lowest recorded abundances also are from shallow water at 5-7 m depth on nearby reefs in Puerto Morelos, Mexico, where only 0.005 individuals occur per 10 m^2 (Briones-Fourzan et al. 2012; Table 3). At St. Thomas, U.S. Virgin Islands, low abundances of 0.14-0.04 individuals per 10 m^2 occur on patch reefs at 6-10 m depth, which receive midday irradiance (PAR) of ~60-160 µmol quanta m^{-2} s^{-1} (Table 1). These data reveal that even shallow reef areas containing *C. gigantea* may be exposed to relatively low irradiance, which is within the range of preferred values of this species. As such, this species appears able to adapt to a wide range of depths and irradiance conditions on coral reefs. More extensive information on depth-related variation in *C. gigantea* abundances and on irradiance levels in the microhabitats where they naturally occur would enhance understanding of their light tolerance levels in the field.

Low sample sizes and wide variation among individuals within treatments may have caused the body size measures examined here (TCSA, WM) to not differ significantly among irradiance treatments. The only substantial change in body size, observed was between Weeks 0 and 2, and may have been linked to changes in the TCSA:WM ratio (Fig. 5). Although we observed a significant change in TCSA:WM ratio only between the medium and low treatments, a larger sample size may have caused the high irradiance treatment to also differ significantly (Fig. 5, Table 1). This is the first report of sea anemones at low irradiance increasing their

surface area more per gram wet mass than do those at high irradiances. Our results indicate that during the initial few weeks of exposure to a high light environment, some anemones decrease their tentacle crown dimensions possibly as a mechanism to shelter their microalgae and compensate for increased irradiance (Fig. 5).

The observed trend of decreasing body size in all experimental treatments, for both body size measures (Fig. 8), may have been caused in part by the feeding regime. The optimal feeding regime leading to growth of Mediterranean sea anemones *Actinia equina* is when polyps are fed to satiation twice each week (Chomsky et al. 2004). In the present study, the once weekly feeding rate may have provided insufficient heterotrophic input for these large anemones. However, this feeding regime was implemented to so that effects of irradiance on photosynthate contribution to host growth could be detected, in anemones that were not already satiated with most of their nutritional needs met via heterotrophy. This experimental design is similar to that employed previously to detect environmental effects on autotrophy in anemones, in that individuals were starved and shrank during treatments, but less so when their microalgae were fertilized with dissolved nitrogen (Roopin and Chadwick 2009). Previous research has shown that many symbiotic cnidarians, including corals and anemones, can fill most of their metabolic needs through autotrophy (Muscatine et al. 1981, Stambler & Dubinsky 1987). However, heterotrophy in the branching coral *Stylophora pistillata* is an important factor in the amount of photosynthate produced through autotrophy (Tremblay et al. 2013), probably through nutritional enhancement of their microalgae. Our results indicate that *C. gigantea* may rely more heavily on heterotrophy than on autotrophy, especially because they are large and fleshy with higher tissue mass per polyp than all other Caribbean anthozoans. Their exposure here to PAR values well below the maxima known for shallow reefs (~1200+ μmol photons m^{-2} s^{-1}, (Lesser 2000, Dixon

et al. 2014) may have contributed to their decline in body size due to lack of translocated photosynthate. However, the anemones at even relatively low irradiance (~300 μmol photons m^{-2} s^{-1}) in our highest-exposure treatment reduced their chl a per cell, microalgal abundances, and body surface areas, more so than did those in the low-exposure treatment, indicating possible effects of photoinhibition and light damage at well below the maxima known for tropical reefs.

Physiological responses of the symbiotic *Symbiodinium* in *C. gigantean* were varied, but generally followed trends reported in the literature (reviewed in Dixon et al. 2014). An initial increase in microalgal abundance under low light indicated that anemones showed a rapid response to irradiance change over two weeks, by hosting significantly more abundant *Symbiodinium* than under high irradiance anemones at Week 2. The observation that microalgal abundance in the low irradiance anemones then decreased and remained static for the remainder of the experiment, indicates that this process was an initial short-term response that did not last. Chlorophyll *a* concentrations showed a likewise rapid response, with high light anemones exhibiting a decrease in chl *a* concentration over the initial two weeks, then subsequently remaining static for the remainder of the experiment. Low irradiance anemones however showed a gradual increase in chl *a*, culminating in a significant difference between low and high irradiance anemones during Week 4. Both of these physiological changes to the *Symbiodinium* indicate their rapid adjustments to the irradiance changes, and may work in concert with each other to enhance photosynthate translocation at low light. The low irradiance anemones appeared to initially increase their abundance of *Symbiodinium* within the tentacle tissues in response to lower irradiance levels, and the *Symbiodinium* then increased their chl *a* concentrations per cell, which may have allowed them to maximize their light capture. The decrease in *Symbiodinium* abundance after this initial increase may have been due to anemone mediation of *Symbiodinium*,

in that anemones may have expelled any damaged *Symbiodinium* to regulate their microalgal density and rid themselves of damaged *Symbiodinium*. Previous studies have proposed this as a mechanism of microalgal cell regulation for many coral species (Titlyanov et al. 1996, Jones & Yellowlees 1997, Baghdasarian & Muscatine 2000, Dimond & Carrington 2008, Fujise et al. 2013). Interestingly, mitotic index decreased in all treatments throughout the experiment, despite static microalgal abundances in both the medium and high treatments and a significant increase in the low treatment. This pattern could have been due to the movement of *Symbiodinium* symbionts from other body areas into the tentacles of the anemones, where light capture occurs more easily, as known for other cnidarian species (Santos et al. 2009).

These results indicate that *C. gigantea* acclimates to a wide range of irradiance via several types of mechanisms. These patterns provide a scientific basis to support aspects of conservation and management of this species. Given the behavioral selection of low irradiance habitats by these anemones, and their ability to acclimate physiologically to low irradiance, they may be able to survive well in the low-light habitats that occur on deep reef slopes. As such, deep reefs may act as a refuge for populations of these sea anemones, as ocean temperatures increase. Although in nature these anemones have been observed mostly in shallow depths (Table 3), this pattern may be an artefact of limited observations on deep coral reefs. The mobility of *C. gigantea*, and their ability to expand and contract into reef holes and crevices may allow these anemones to survive on shallow reefs where irradiance levels are high, in that behaviorally they are able to reduce their exposure to high light even in shallow water.

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Chapter III

Conclusions and recommendations

In conclusion, this study highlights the ability of the *Condylactis gigantea* holobiont to behaviorally and physiologically adapt to varying irradiance levels. By changing the abundance of *Symbiodinium* and the amount of chl *a* within their microalgal cells, and manipulating their surface area to wet mass ratio, these anemones can adapt to a wide range of irradiance environments and depths on coral reefs, thereby expanding their optimal tolerance range within tropical marine environments.

Based on these experimental results, we propose several recommendations concerning the conservation management of this species, and the aquaculture of individuals for the ornamental marine aquarium trade. We recommend that management for this species should focus on their preservation in highly rugose and/or deep coral reef habitats. Previous research has shown that throughout the Caribbean Sea, a drastic decline in rugose reef habitat has occurred over 40 years between 1969-2008 (Alvarez-Filip et al. 2009). Over this 40-year time period, the least rugose reefs began to dominate the aquatic landscape; ~75% of reefs had a rugosity rating of less than 1.5 in 2008, compared to ~20% in the 1970's (Alvarez-Filip et al. 2009). Rugose reefs have highly three-dimensional structure in which these anemones may be able to optimally use their behavioral and physiological responses to manipulate the effects of the light environments they occupy. Variation in their levels of expansion versus contraction in holes and

crevices in coral reefs can allow individuals of *C. gigantea* to reduce irradiance-related stress, allowing them to better respond to other environmental stressors and reducing their chances of light-enhanced bleaching. The deep slope coral reef habitat and "mesophotic" reef habitat may be refuges for *C. gigantea* as global warming continues. Our results indicating that *C. gigantea* can alter their surface area to wet mass ratio show a unique mechanism by which these anemones modulate light exposure to their microalgae, in that their body shape changes potentially can allow increased light capture by resident *Symbiodinium* under low light, or shield their microalgae from damage under high light. Having the ability to rely more on heterotrophy under low light may make this species of sea anemone adept at sustaining and flourishing in deep reef habitats, and allow deep populations potentially to seed shallow water habitats, as proposed for some coral species (Bongaerts et al. 2010).

The results presented here also contribute methods for the aquaculture of individuals for the ornamental aquarium trade, in that they can be used to enhance the culture conditions for *C. gigantea*. Based on the narrow range of low irradiance levels that these sea anemones behaviorally select in aquariums, we recommend that aquarists tailor the lighting environment so that *C. gigantea* tentacles are exposed to ~40-60 µmol quanta m^{-2} s^{-1} of PAR. We also recommend high rates of heterotrophic feeding of these anemones, at least 2x per week to satiation or even more frequently, so as to enhance their growth and survival in commercial culture and in home aquariums. Increased health and survival of *C. gigantea* in aquariums likely will decrease the demand for wild-collected individuals and thus reduce the collection pressure on natural populations. We conclude that the variety of behavioral and physiological acclimation mechanisms of *C. gigantea* revealed here contribute to better understanding of the ecology of

natural populations of this important coral reef anthozoan, as well as providing a biological basis for improved conservation management and aquarium culture.

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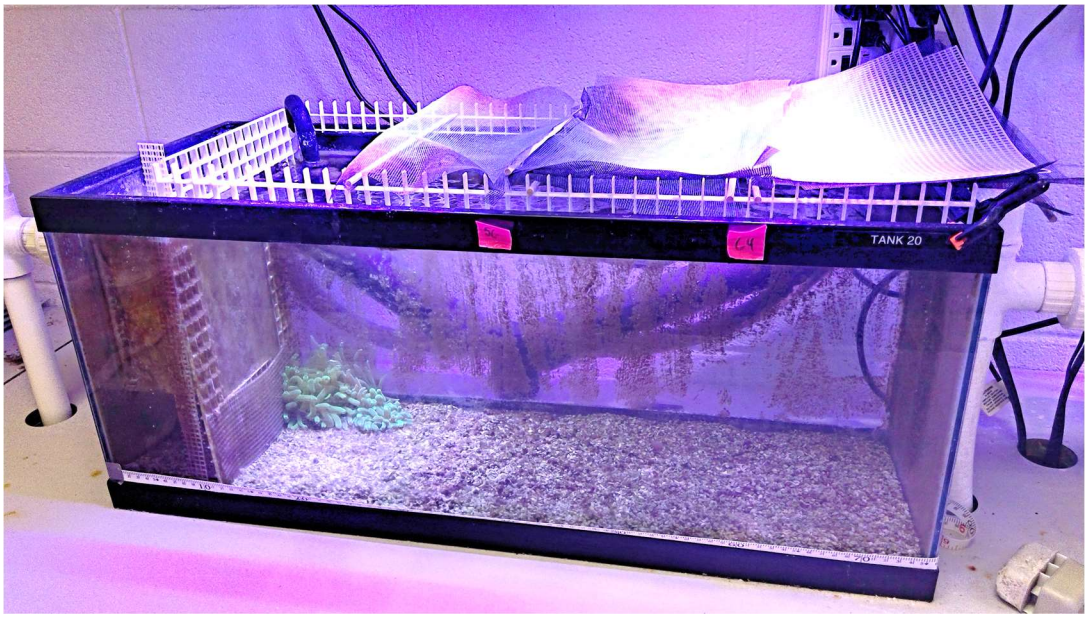

Figure 1a Light tunnel set up for phototaxis experiments. Note the mesh covers over the tank top to create a gradient of low to high irradiance from right to left. Note also the rosetip sea anemones *Condylactis gigantea* visible at left; they are not in their typical positions as recorded in most trial replicates, in which the majority of anemones selected low-irradiance locations in the trial tanks (see Figs. 2-4).

Figure 1b Chambered tank set up for 6-wk growth experiment. Note that irradiance levels differed widely among the 4 chambers, and that one individual rosetip sea anemone *Condylactis gigantea* occupied each chamber. Irradiance treatments were assigned randomly to chamber positions in each tank. This is the same tank as the one visible in Fig. 1a, but configured for the growth experiment instead of as a light tunnel for the phototaxis experiment.

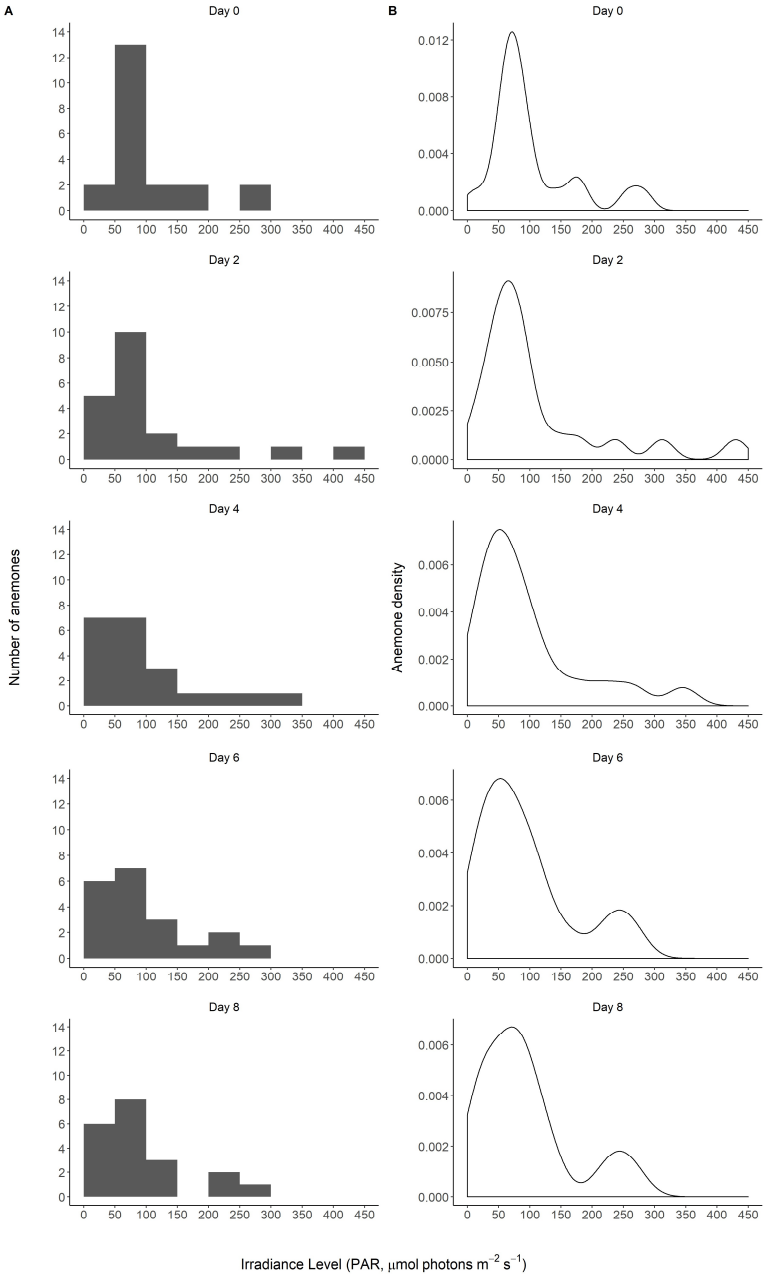

Figure 2 Variation in the distribution of rosetip sea anemones *Condylactis gigantea* (N = 21) among levels of irradiance (photosynthetically active radiation; PAR) within experimental light tunnels, every other day for 8 days during the first phototaxis experiment. (**A**) Number of individuals at each irradiance level. (**B**) Kernel density plots of anemone densities at each irradiance level. Data not shown for days 1, 3, 5, and 7, to reduce repetition.

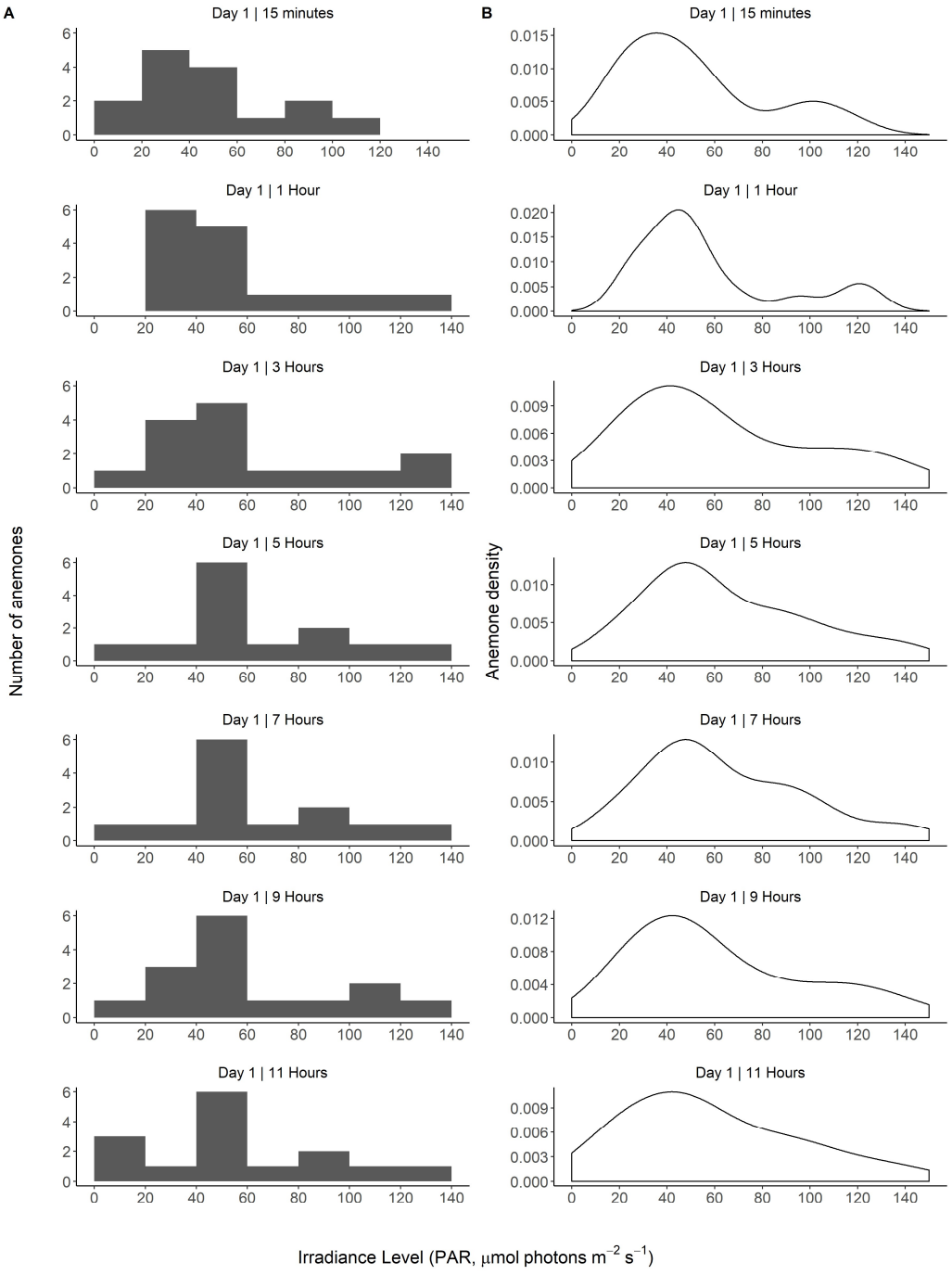

Figure 3 Variation in the distribution of rosetip sea anemones *Condylactis gigantea* (N = 15) among levels of irradiance (photosynthetically active radiation; PAR) within experimental light tunnels, every other hour or more during the first day of the second phototaxis exeriment. (**a**) Number of individuals at each irradiance level (**b**) Kernel density plots of anemone densities at each irradiance level. Data not shown for hours 2, 4, and 6, to reduce repetition.

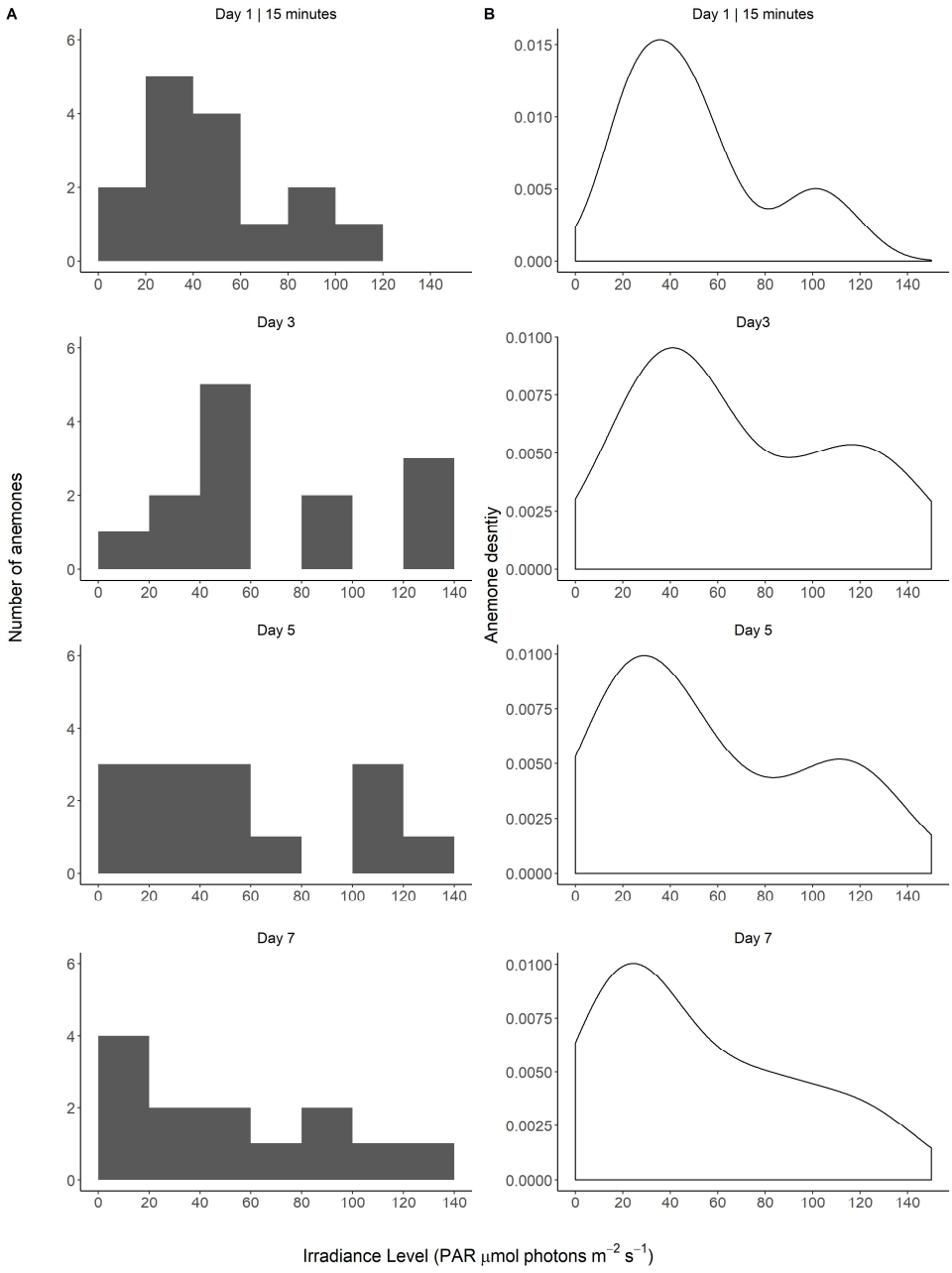

Figure 4 Variation in the distribution of rosetip sea anemones *Condylactis gigantea* (N = 15) among levels of irradiance (photosynthetically active radiation; PAR) within experimental light tunnels, every other day for 7 days in the second phototaxis exeriment. (**a**) Number of individuals at each irradiance level. (**b**) Kernel density plots of anemone densities at each irradiance level. Data not shown for days 2, 4, and 6, to reduce repetition.

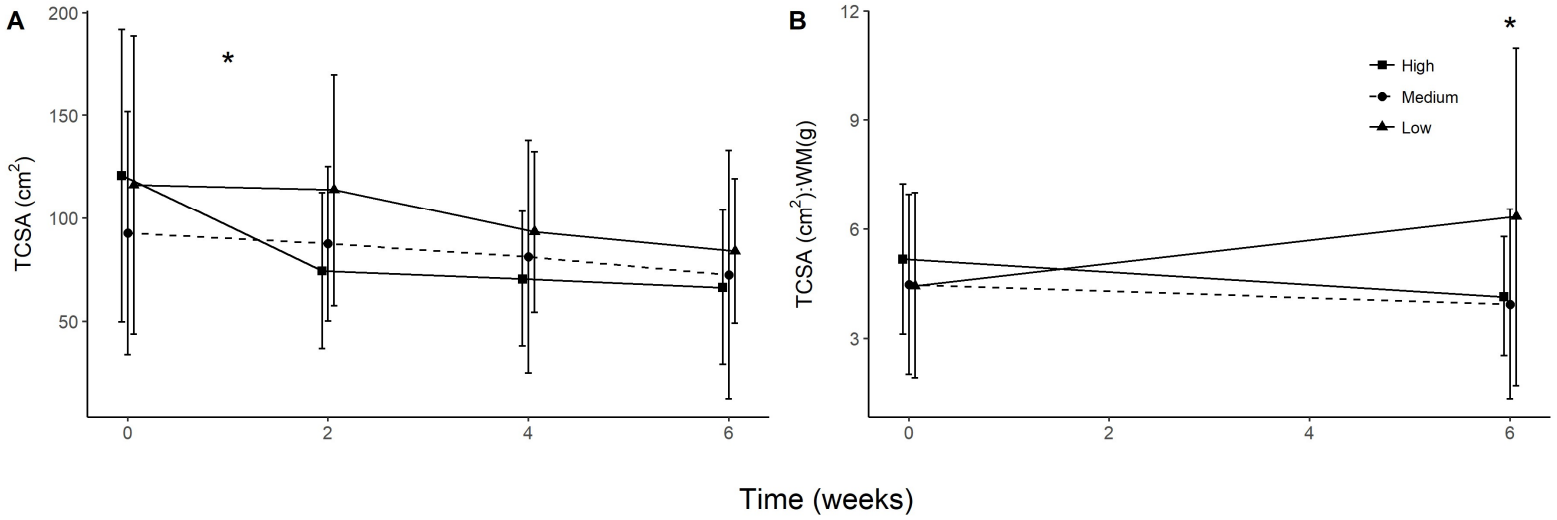

Figure 5 Variation in the body size relationships of rosetip sea anemones *Condylactis gigantea* among 3 irradiance treatments (Low, Medium, and High) during 6 weeks under laboratory conditions. (**a**) Tentacle crown surface area (TCSA); (**b**) TCSA:Wet Mass (WM) ratio. Wet mass was measured only at the start and end of the experiment in order to reduce stress on the anemones. Data are shown as means ± 1 SD, for anemones exposed to Low irradiance (~50 µmol quanta m^{-2} s^{-1}; N = 13-16), Medium irradiance (~150 µmol quanta m^{-2} s^{-1}; N = 12-16), or High irradiance (300+ µmol quanta m^{-2} s^{-1}; N = 12-16). Asterisks at weeks indicate significant differences among treatments, while asterisks between weeks indicate significant interactions between treatment and time (Table 1).

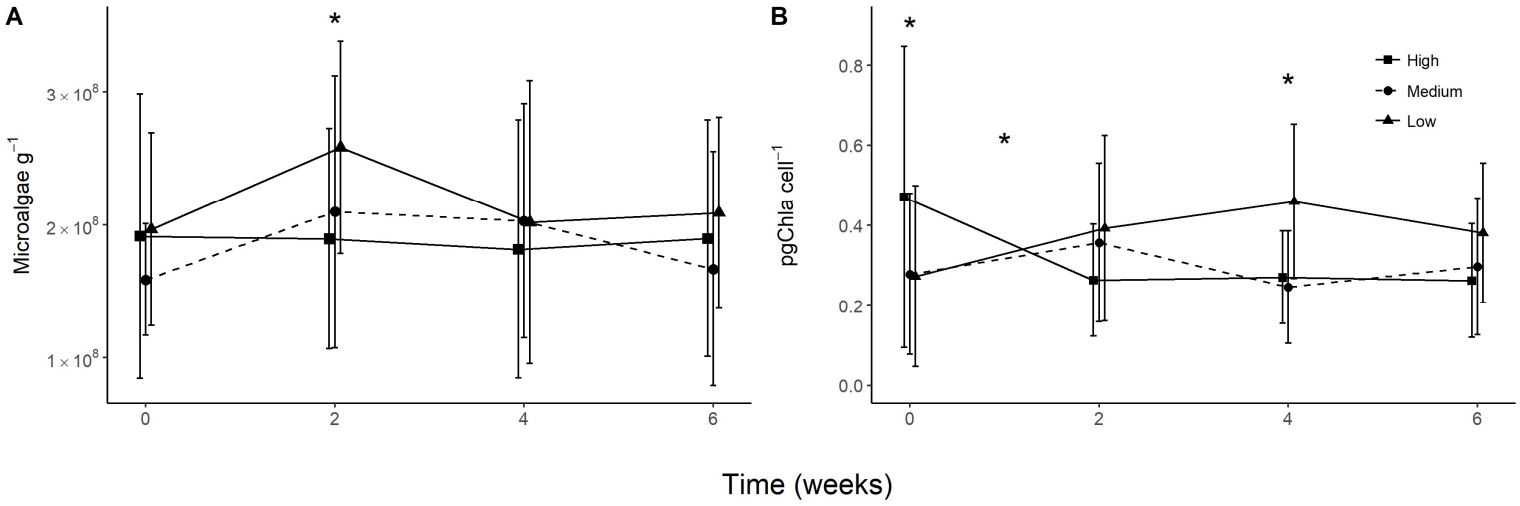

Figure 6 Variation in *Symbiodinium* characteristics of rosetip sea anemones *Condylactis gigantea* among 3 irradiance treatments (Low, Medium, and High) during 6 weeks under laboratory conditions. (**a**) Abundance of microalgal (*Symbiodinium*) cells per gram wet mass (WM) of sea anemone tentacles; (**b**) Chlorophyll *a* concentration per microalgal cell. Data are shown as means ± 1 SD, for anemones exposed to Low irradiance (~50 µmol quanta m^{-2} s^{-1}; N = 13-16), Medium irradiance (~150 µmol quanta m^{-2} s^{-1}; N = 12-16) individuals, or High irradiance (300+ µmol quanta m^{-2} s^{-1}; N = 12-16). Asterisks at weeks indicate significant differences among tretments, while asterisks between weeks indicate significant interactions between treatment and time (Table 1).

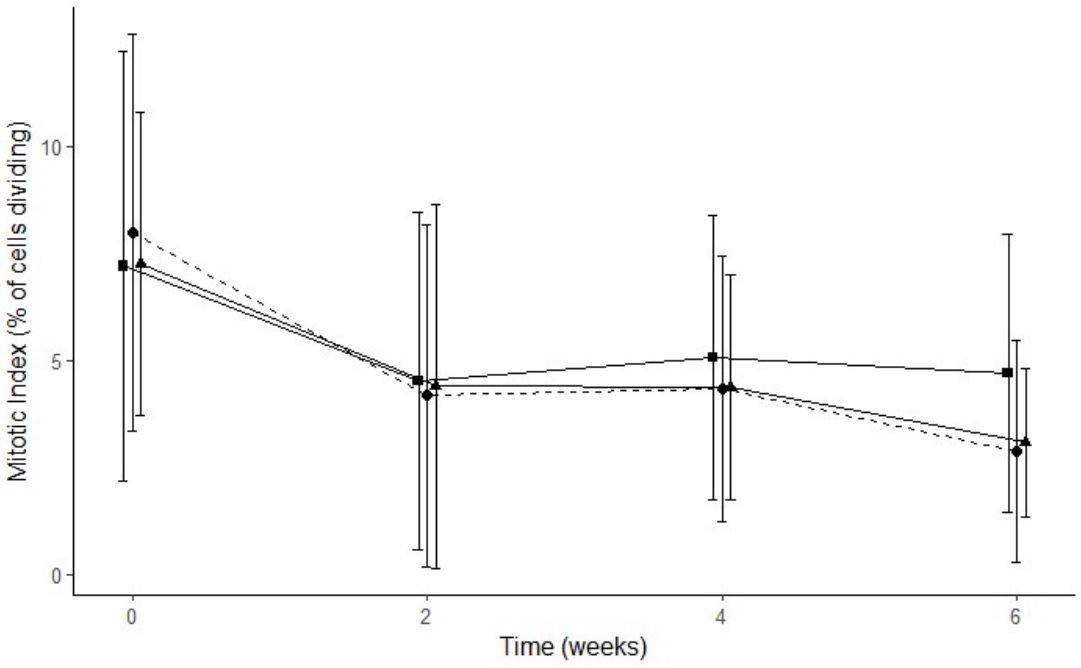

Figure 7 Variation in the mitotic index of *Symbiodinium* cells in rosetip sea anemones *Condylactis gigantea* among 3 irradiance treatments (Low, Medium, and High) during 6 weeks under laboratory conditions. Data are shown as means ± 1 SD, for anemones exposed to Low irradiance (~50 µmol quanta m^{-2} s^{-1}; N = 14-16), Medium irradiance (~150 µmol quanta m^{-2} s^{-1}; N = 12-16), or High irradiance (300+ µmol quanta m^{-2} s^{-1}; N = 12-16) individuals. Asterisks indicate significant differences in estimates of effect between treatments (Table 1).

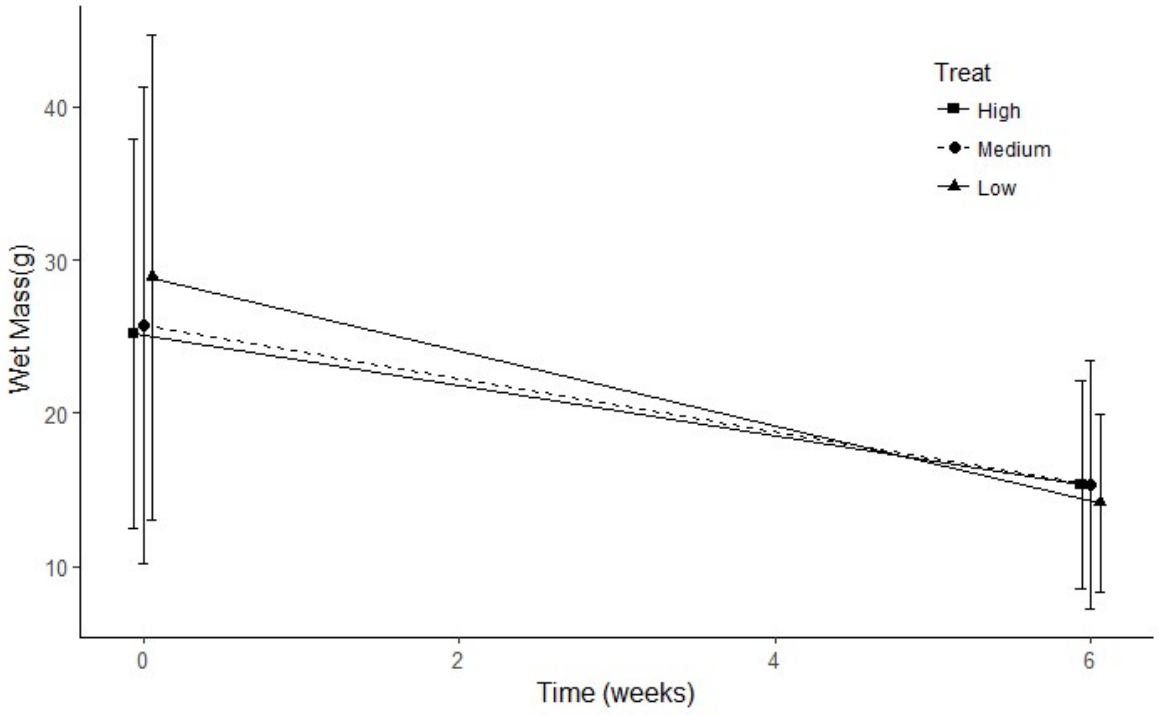

Figure 8 Variation in the wet mass of rosetip sea anemones *Condylactis gigantea* among 3 irradiance treatments (Low, Medium, and High) between Weeks 0 and 6 under laboratory conditions. Wet mass was measured only at the start and end of the experiment in order to reduce anemone stress. Data are shown as means ± 1 SD, for anemones exposed to Low irradiance(~50 µmol quanta m^{-2} s^{-1}; N = 14-16), Medium irradiance (~150 µmol quanta m^{-2} s^{-1}; N = 12-16), or High irradiance (300+ µmol quanta m^{-2} s^{-1}; N = 12-16).

Table 1. Variation in physiological parameters of Condylactis gigantea between treatments throughout a 6 week laboratory experiment. Estimates of effect are shown for treatment effects (β1), bold p-values show significant effects of treatment.

Parameter	Treatment	β_1	95% CI	*p*
TCSA	Low vs. High			
	0	-5.02	±37.53	0.7903
	2	35.69	±38.58	0.0706
	4	16.73	±39.62	0.4026
	6	24.66	±41.20	0.2373
	Medium vs. High			
	0	-27.62	±37.41	0.1464
	2	3.802	±39.36	0.8476
	4	0.79	±40.56	0.4026
	6	4.805	±41.76	0.819
	Medium vs. Low			
	0	22.61	±37.52	0.2343
	2	31.89	±38.43	0.1036
	4	15.94	±39.50	0.4236
	6	19.85	±41.08	0.3388
TCSA:WM	Low vs. High			
	0	-0.72	1.99	0.4730
	6	2.18	2.22	0.0553
	Medium vs. High			
	0	-0.69	1.99	0.4906
	6	-0.21	2.301	0.8545
	Low vs. Medium			
	0	-0.029	1.99	0.9773
	6	2.39	2.22	**0.0362**
chl *a*	Low vs. High			
	0	-0.2	±0.15	**0.0092**
	2	0.13	±0.15	0.0832
	4	0.19	±0.15	**0.0153**
	6	0.12	±0.16	0.137
	Medium vs. High			
	0	-0.19	±0.15	**0.012**
	2	0.092	±0.15	0.2167
	4	-0.023	±0.15	0.7655
	6	0.042	±0.17	0.6119
	Low vs. Medium			
	0	-0.0076	±0.15	0.9173
	2	0.038	±0.15	0.6007
	4	0.22	±0.15	**0.0063**
	6	0.079	±0.16	0.3295
Microalgal abundance	Low vs. High			
	0	7.80×10^6	6.14×10^7	0.8005
	2	6.92×10^7	6.23×10^7	**0.0311**
	4	2.20×10^7	6.37×10^7	0.4925
	6	8.33×10^6	6.62×10^7	0.8024
	Medium vs. High			
	0	-3.27×10^7	6.12×10^7	0.2909
	2	2.13×10^7	6.20×10^7	0.4963
	4	2.34×10^7	6.36×10^7	0.4660
	6	-2.58×10^7	6.78×10^7	0.4504
	Low vs. Medium			
	0	4.05×10^7	6.15×10^7	0.1943
	2	4.79×10^7	6.15×10^7	0.1259
	4	-1.32×10^6	6.29×10^7	0.9666
	6	3.41×10^7	6.61×10^7	0.3073
Mitotic index	Low vs. High			
	0	0.057	±2.61	0.9651
	2	-0.22	±2.64	0.8704
	4	-0.54	±2.70	0.6881
	6	-1.14	±2.79	0.4158
	Medium vs. High			
	0	0.78	±2.60	0.5518
	2	-0.26	±2.63	0.8424
	4	-0.65	±2.69	0.6299
	6	-1.92	±2.84	0.1833
	Low vs. Medium			
	0	-0.72	±2.61	0.5838
	2	0.046	±2.61	0.9720
	4	0.107	±2.67	0.9363
	6	0.77	±2.78	0.5805

Table 2. Variation in physiological parameters of *Condylactis gigantea* between treatments throughout a 6 week laboratory experiment. Estimates of effect are shown for interactions (β3), bold p-values show significant interactions.

Parameter	Treatment x Time	β_3	95% CI	*p*
TCSA	Low vs. High			
	0-2	-40.71	±40.67	**0.0477**
	2-4	18.97	±41.70	0.3652
	4-6	-7.93	±43.64	0.7170
	Medium vs. High			
	0-2	-31.43	±41.83	0.1358
	2-4	3.02	±43.64	0.8903
	4-6	-4.01	±44.96	0.8584
	Low vs. Medium			
	0-2	-9.29	±40.52	0.6476
	2-4	15.95	±42.17	0.4510
	4-6	-3.91	±43.86	0.8588
TCSA:WM	Low vs. High			
	0-6	2.903	±2.98	0.0595
	Medium vs. High			
	0-6	0.48	±3.044	0.7542
	Low vs. Medium			
	0-6	2.42	±2.98	0.1131
chl *a*	Low vs. High			
	0-2	-0.33	±0.18	**0.0005**
	2-4	-0.064	±0.13	0.5
	4-6	0.073	±0.20	0.4603
	Medium vs. High			
	0-2	-0.28	±18	**0.0026**
	2-4	0.12	±0.23	0.2205
	4-6	-0.065	±0.2007	0.5171
	Low vs. Medium			
	0-2	-0.046	±0.18	0.6129
	2-4	-0.18	±0.19	0.0563
	4-6	0.14	±0.20	0.1615
Microalgal abundance	Low vs. High			
	0-2	-6.13×10^7	$\pm 6.32 \times 10^7$	0.0546
	2-4	4.71×10^7	$\pm 6.45 \times 10^7$	0.1463
	4-6	1.37×10^7	$\pm 6.76 \times 10^7$	0.686
	Medium vs. High			
	0-2	-5.39×10^7	$\pm 6.37 \times 10^7$	0.093
	2-4	-2.09×10^5	$\pm 6.45 \times 10^7$	0.9485
	4-6	4.91×10^7	$\pm 6.93 \times 10^7$	0.1588
	Low vs. Medium			
	0-2	5.39×10^7	$\pm 6.37 \times 10^7$	0.093
	2-4	4.92×10^7	$\pm 6.34 \times 10^7$	0.1234
	4-6	-3.54×10^7	$\pm 6.72 \times 10^7$	0.2935
Mitotic index	Low vs. High			
	0-2	0.27	±2.47	0.8248
	2-4	0.33	±2.52	0.795
	4-6	0.60	±2.64	0.6507
	Medium vs. High			
	0-2	1.041	±2.49	0.4053
	2-4	0.39	±2.52	0.7584
	4-6	1.27	±2.71	0.3512
	Low vs. Medium			
	0-2	-0.77	±2.44	0.5311
	2-4	-0.0609	±2.48	0.9609
	4-6	-0.67	±2.62	0.6118

Table 3. Distribution and abundance patterns of *Condylactis gigantea*

Location	Habitat	Depth (m)	Abundance (anemone/10m^2)	Irradiance (µmol photons m^{-2} s^{-1})	N	Source/notes
Belize, Glover's Reef	Reef lagoon	1-3	-	-	100	Blanquet et al. (1980)
Bermuda	-	1-7	-	-	29	Crawford (1992)
Bermuda	Rubble	0-6	0.5	-	41	Nizinksi (1989), 41 indv. Within 8 x100m area
Bermuda, Tyne's Reef	-	6	-	-	31	Venn (2008)
Jamaica, Discovery Bay	-	27-37	-	-	-	Colin (1973)
Jamaica, Discovery Bay	-	1-3	-	-	6	Kellog and Patton (1983)
Mexico, Akumal	Patch reefs	1-4	0.95 ±1.14	-	128	Columbara (2017), 15 quadrants examined
Mexico, Puerto Morelos	Back reef	1.8-2	0.03	-	85	Briones-Fourzan et al. (2012)
	Reef channels	5-7	0.005	-	14	Briones-Fourzan et al. (2012)
	Fore reef	8-10.2	0.03	-	85	Briones-Fourzan et al. (2012)
United States, Dry Tortugas	Spur and groove reef	6-15	0.17	-	1	*Miller et al. (2008a); estimated from data
	Reef terrace	15-21	0.17	-	3	*Miller et al. (2008a); estimated from data
United States, Dry Tortugas	-	3-7	-	-	8	Hanlon (1986)
United States, Upper Florida Keys	Backreef to patch reefs	3.1-6	0.1 - 0.2	-	11	*Miller et al. (2008b); estimated from data
	Fore reef	9-11.6	0	-	0	*Miller et al. (2008b); estimated from data
United States, Middle Florida Keys	Backreef to patch reefs	3.8-5.7	0.02 - 0.2	-	8	*Miller et al. (2008b); estimated from data
	Fore reef	9.5-10.7	0.01	-	1	*Miller et al. (2008b); estimated from data
United States, Lower Florida Keys	Backreef to patch reefs	3.1-8.4	0.02-0.04	-	4	*Miller et al. (2008b); estimated from data
	Fore reef	9-11.6	0.01	-	1	*Miller et al. (2008b); estimated from data
United States, Virgin Islands	Inshore patch reef	6	0.14	59	4	N.E. Chadwick, unpublished data
	Offshore patch reef	10	0.004	195	5	N.E. Chadwick, unpublished data

*Miller S, Chiappone M, Rutten L, (2008a). Quick look report (Tortugas). VI. Anemones and corallimorpharians. Coral Reef Monitoring and Assessment Reports, University of North Carolina at Wilmington, USA. http://people.uncw.edu/millers/documents/Tortugas_2008_Quicklook_Part-6_Anemones_and_corallimorpharians.pdf

*Miller S, Chiappone M, Rutten L, (2008b). Quick look report (Florida Keys). VI. Anemones and corallimorpharian density. Coral Reef Monitoring and Assessment Reports, University of North Carolina at Wilmington, USA. http://people.uncw.edu/millers/documents/Keyswide_2008_Quicklook_Part6_Cnidarians.pdf

Note: For this table, data from the reports by Miller et al. were used from only the first year (2008) of 3 years of reports. For details see: http://people.uncw.edu/millers/CoralReef_QuickLooks.htm